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Original Article

Effect of *Bacillus subtilis* as a Probiotic on the Productive and Physiological Performance of Broilers

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Abstract

Broiler chickens are reared in relatively dense colonies or flocks under intensive production environments to reach opportunities for financial efficiency. A plethora of variables, including overcrowding, immunization, and transportation, can cause serious stress. This study aimed to determine changes in the productive and physiological performance of broiler chickens (Rose 308) after adding different concentrations of the probiotic liquid *Bacillus subtitles* (*B. subtitles*) to their diet. A total of 120 birds of the hatching age (Ross 308) were divided into four groups, each of which had three replicates. Each repetition included 10 birds the experiment lasted for five weeks after it commenced. The implementation of the diets was as follows: the control group received a regular diet without probiotic, and the probiotic-treated groups were supplemented with different concentrations of *B. subtilis* BSW equal to 1×10^4, 1×10^6, and 1×10^8 CFU/gm diet. At the end of the trial, the results indicated a significant improvement in both the live body weight and the efficiency of feed conversion when adding different levels of probiotics to the broilers diet. Furthermore, the findings showed a significant increase ($P\leq0.05$) in the total serum protein, serum albumin, and serum globulin for the treated groups, compared to the control group at the age of five weeks. It is concluded that the dietary supplementation of *B. subtilis* BSW to the diet of broilers significantly improved their growth performance, in comparison with the control group. Instead, the treated groups exhibited a substantial increase in the total serum protein, serum albumin, and serum globulin, as compared to the control group. These findings suggest that *B. subtilis* BSW strain possesses probiotic properties, making it a suitable supplement for the poultry diet.

Keywords: Albumin, Chicken, Globulin, Probiotics, Protein, Weight

1. Introduction

Broiler chickens are reared in relatively dense colonies or flocks under intensive production environments to reach opportunities for financial efficiency. A plethora of variables, including overcrowding, immunization, and transportation, can cause serious stress (1). It has become clear that the selection of approaches for increasing body weight in broiler chickens contributes to reducing the quality of poultry products due to the increase in fat mass. Therefore, the high of percentage fat content in broiler carcasses turned out to be a problem not only in terms of customer satisfaction but also in terms of the processing loss (2). Modern broiler production may achieve more effective use of feed, which correlates with an increased weight gain and a shorter rearing time, compared to earlier production settings. To ensure this great efficiency is not adversely affected, it is critical to maintain the animals' guts in good health (3).

However, probiotics are recognized as live microorganisms that greatly impact the general body health and organ functioning by improving the activity of the gastrointestinal tract, as well as the intestines. The *B. subtilis*, a prominent probiotic species, is considered one of the healthiest bacteria to promote nutritional digestion and absorption in the host's body. Live bacterial cells added to feed may increase broiler chickens' digestibility and performance qualities when used as a poultry growth promoter by generating optimal growth conditions (4). In particular, they can enable the organs to stimulate the body's immunity against pathogens, which in turn enhances the performance and productive qualities of birds. Probiotics have been used widely as a feed additive in poultry diets (5). Probiotics can enhance broiler chickens' body weight and their weight gain, in addition to reducing their feed consumption, as well as mortality (6, 7). It has been observed that probiotics can provide an improvement in the feed conversion ratio as well (8). They can also increase broiler chickens' body weight by reducing the number of pathogenic bacteria that invade the gastrointestinal tract (9).

Nevertheless, *Bacillus* species (spp.), *Lactobacillus* spp., *Aspergillus* spp., *Saccharomyces* spp., and *Streptococcus* spp., among many other microbial spp., have been utilized as probiotics in poultry feed to improve their productivity (10). *Bacillus* spp. are of importance in feed additives since they are persistent bacteria that could produce spores, as well as a variety of enzymes, targeting different nutrients, including protease, lipase, and amylase, in addition to the ability to synthesize antimicrobial agents (11, 12). The most frequent method of administering probiotics in the poultry diet is through feed, although there are a variety of other options, such as gavage, sprays, pellets, pills, capsules, or powder packs (13). All of these additives can improve the productive performance of birds, their weight gain, and feed efficiency (14, 15).

Therefore, the main objective of this study was to improve the performance of birds in terms of increasing weight and lowering feed consumption, as well as to demonstrate the probiotic properties of the local isolate *B. subtilis* BSW and its potential use as an effective poultry growth promoter.

2. Materials and Methods

2.1. Birds Management

A total of 120 Ross (308) broiler chickens, one day old, were divided into four groups, each of which had three replicates. Each repeater included 10 birds. The experiment lasted for five weeks, in which the birds were fed and water adds *libitum*.

2.2. Bacterial Strain and Diet

The *B. subtilis* BSW, which is the probiotic bacterium used in this study, has been previously isolated and identified in the lab of biotechnology belonging to the Department of Food Sciences at the College of Agriculture, University of Basrah, Basra, Iraq. It was then transferred to the gene bank (Accession number: OL984047) where it was cultivated and counted using the spread plate method on the standard nutrient agar. Before usage, probiotic stock culture was maintained at -20˚C in a powdered skim milk suspension with 25% glycerol. Afterward, the *B. subtilis* BSW strain was cultivated in a nutrient broth at 37˚C in a shaking incubator (Sartorius-Certomat IS, Germany) at 150 rpm for 24 h. The cells were centrifuged (Hermle Labor Technik GmbH, Germany) at 2,000 g and 4˚C for 10 min, washed three times with PBS (pH 7.2), and resuspended in the same buffer for diet preparations. Afterword, *B. subtilis* BSW cells were suspended in skim milk powder (2×10^{10} CFU/gm) and added to the basal diet (Table 1) of the treated groups: low group (1×10^{4} CFU/gm of feed), middle group (1×10^{6} CFU/gm of feed), and high group (1×10^{8} CFU/gm of feed). To achieve these final concentrations, *B. subtilis* BSW was gradually introduced to the diets. Plate counting on the nutrient agar was used to determine and modify the number of probiotic bacteria in each diet.

Table 1. Bird feed ingredients

Ingredients (%)	Starter	Grower
	(1-21 days)	(22-35 days)
Ground yellow corn 8.5%	62.04	64.54
Soybean meal 44%	24.14	23.82
Gluten of corn 60%	9.1	4.58
Vegetable fat	0.13	3.3
Calcium phosphate	2.10	1.6
Calcium carbonate	1.5	1.12
Vitamins and minerals	0.2	0.2
Salt	0.4	0.4
Methionine	0.13	0.20
Lysine	0.26	0.24
Total	100	100
Calculated chemical analysis		
Crude protein	21.01	18.01
ME. Cal/Kg feed	2986.70	3176.00
C/P ratio	142.16	176.35
Calcium	1.04	0.90
Phosphor (available)	0.50	0.47
Lysine	1.20	1.06
Methionine	0.52	0.50
Cyst. + methionine	0.89	0.82

According to the NRC (1994), each 3Kg of vitamin and mineral mixture contains vitamin A 10.000.000 IU, vitamin D3 2.000.000 IU, vitamin E 10.000 mg, vitamin K3 1.000 mg, vitamin B1 1.000 mg, vitamin B2 5.000 mg, vitamin B6 1.500 mg, vitamin B12 10 mg, Niacin 20.000 mg, Pantothenic Acid 10.000 mg, Folic acid 1.000 mg, Biotin 50 mg, Choline Chloride 500.000 mg, Copper 4.000 mg, Iodine 300 mg, Iron 30.000 mg, Manganese 60.000 mg, Zinc 50.000 mg, Cobalt 100 mg, and Selenium 100 mg.

2.3. Performance

Regarding body weights, feed consumed, and feed conversion efficiency, data were recorded for all birds in the experiment in weeks 1, 2, 3, 4, 5, and 6. Body weight and feed intake were calculated according to Al-Fayyad and Nagy (16), whereas feed conversion efficiency was calculated according to Al-Fayyad and Nagy (16).

2.4. Blood Sampling

At the end of the experiment, 12 birds were selected from all groups, (three birds from each group), and 3 ml of blood was drawn from the axillary vein. It was poured into anti-coagulation tubes and centrifuged for 20 min at 4˚C. Subsequently, serum samples were withdrawn and then emptied into the Eppendorf sterilized tubes with a volume of 1.5 ml. Finally, they were thawed at 4˚C before performing the analysis.

2.5. Statistical analysis

All data were subjected to the analysis of variance (One-Way ANOVA) by a completely random design using the SAS (Duncan, 1955).

3. Results

Tables 2, 3, and 4 showed the impact of probiotic *B subtilis* BSW with (1×10^4 CFU/gm, 1×10^6 CFU/gm, and 1×10^8 CFU/gm) on the weekly live body weight, the amount of weekly feed intake, and the efficiency of feed conversion. For the treated groups, there were no significant differences in body weight. However, both tables 3 and 5 showed that adding different levels of probiotics to the treated groups' diet led to a significant increase in their weekly live body weights, as well as an improvement in the efficiency of food conversion for them, compared to the control group. These results also indicated that the treated groups had no significant effects on the mentioned variables. However, the experimental group fed with a 1×10^8 CFU/gm feed probiotic/gm diet showed a significant increase ($P\leq0.05$) in their body weight, compared to the control group in the third week. Within the sixth week of the experiment, the findings revealed a slight yet not significant improvement in the efficiency of feed conversion in the group fed with a 1×10^6 CFU/gm diet and 1×10^8 CFU/gm diet, compared to the control group.

Table 2. Impact of adding probiotics to birds' diet on their body weight (gm)

Groups	Age (week)					
Bacillus subtilis BSW (CFU/gm dite)	First	Second	Third	Fourth	Fifth	Sixth
Control	$78.41^1\pm0.88$	198.98±2.85	$418.69^b\pm9.71$	816.03±15.75	1320.99±24.10	1896.08±34.53
1×10^4	78.33±1.13	202.28±4.53	$442.38^{ab}\pm9.49$	833.46±15.23	1347.29±21.07	1925.79±28.97
1×10^6	78.48±1.21	201.94±3.51	$441.95^{ab}\pm7.69$	798.91±13.19	1317.69±18.68	1910.58±27.46
1×10^8	78.38 ±1.08	208.66±3.30	$456.95^a\pm7.48$	821.88±14.00	1374.02±19.98	1968.83±25.93

***** Different letters, vertically and horizontally, mean that there are significant differences at ($P\leq0.05$) significance level

Table 3. Impact of adding probiotics to birds' diet on their feed intake (gm)

Groups	Age (week)				
Bacillus subtilis BSW (CFU/gm dite)	Second	Third	Fourth	Fifth	Sixth
Control	219.83[b1]±11.58	640.50±10.55	894.50[ab]±10.69	1133.08[a]±18.74	1183.75[b]±11.28
1×10[4]	233.50[b]±8.25	660.16±8.77	914.53[a]±14.77	1074.29[b]±16.76	1271.98[a]±39.71
1×10[6]	230.50[b]±5.07	648.50±6.59	863.50[b]±4.15	1134.03a±5.75	1206.80[ab]±2.74
1×10[8]	275.50[a]±11.96	634.75±4.24	876.57[b]±3.01	1150.35[a]±15.30	1183.75[b]±12.73

* *Different letters, vertically and horizontally, mean that there are significant differences at ($P \leq 0.05$) significance level

Table 4. Impact of adding probiotics to birds' diet on feed conversion (gm feed/gm weight gain)

Groups	Age (week)				
Bacillus subtilis BSW (CFU/gm dite)	Second	Third	Fourth	Fifth	Sixth
Control	1.08[b]±0.05	2.04±0.09	2.13±0.02	2.17±0.04	2.13±0.01
1×10[4]	1.13[ab]±0.04	2.01±0.04	2.14±0.04	2.13±0.02	2.16±0.01
1×10[6]	1.12[b]±0.03	1.97±0.02	2.12±0.02	2.17±0.01	2.16±0.02
1×10[8]	1.29[a]±0.07	1.97±0.02	2.07±0.01	2.12±0.01	2.16±0.04

* Different letters, vertically and horizontally, mean that there are significant differences at ($P \leq 0.05$) significance level

Table 5. Impact of adding probiotics to birds' diets on biochemical blood parameters

Group	Age (week)					
	Fifth			Sixth		
Bacillus subtilis BSW (CFU/gm diet)	Total Protein	Albumin	Globulin	Total Protein	Albumin	Globulin
Control	7.74[b1]±0.07	3.18[c]±0.04	4.21[c]±0.05	7.58[b]±0.07	3.45[b]±0.04	4.11±0.06
1×10[4]	7.83[b]±0.07	3.41[ab]±0.07	4.40[b]±0.07	7.90[a]±0.09	3.58[ab]±0.06	4.13±0.19
1×10[6]	8.07[a]±0.03	3.28[bc]±0.04	4.79[a]±0.06	7.90[a]±0.08	3.80[a]±0.15	4.16±0.05
1×10[8]	7.91[ab]±0.06	3.51[a]±0.04	4.71[a]±0.05	7.71[ab]±0.01	3.45[b]±0.04	4.25±0.07

* Different letters, vertically and horizontally, mean that there are significant differences at ($P \leq 0.05$) significance level

4. Discussion

The obvious improvement in the weekly live body weight and feed conversion efficiency of groups treated with different levels of the probiotic strain may be attributed to several reasons, including the faster rate of transporting nutrients from the intestine into the bloodstream. This, in fact, happens as a result of reducing the thickness of the circular muscle and the mucous layers of the duodenum in the treated groups, compared to the control group, as described by Izzuddiyn, Busono (17). Furthermore, it has been reported that in the probiotic-treated groups, the height and width of the villi were higher, whereas the villous area was less, compared to the control group. Such indications showed that the absorption was better in the treated groups because they comprise a larger surface area, compared to the existing ones in the control group (18, 19). It was also revealed that probiotics can directly and positively affect the hosts' health when ingested through the digestive system, which was beneficially reflected in the studied traits (6, 20). Line, Bailey (21) signified that the improvement in the performance of broiler chickens, as a result of adding probiotics supplements, is due to regulating metabolic reactions, which in turn stimulate internal enzymes, antimicrobial substances, and vitamin production Santin, Maiorka (22) interpreted the ability of probiotics to strengthen the natural defenses of the host as a bioregulator for the intestinal microbiota, stimulating the immune response, and increasing resistance to colonization. Nonetheless, the findings of the present study demonstrated that probiotics have the capacity to increase amino acid digestion. In terms of the probiotic effect of supplementation with *B. subtilis* BSW in weeks five and six on the total protein in the blood serum albumin and serum globulin (Table 5), the results showed a high significance in serum protein, as well as albumin and globulin, of the treated groups in the fifth week of the experiment, compared to that in the control group. This increase is probably due to the effect of probiotic bacterium *B. subtilis* BSW on protein metabolism, which is consistent with the slight increase in the body weights of the treated groups. The results showed that the concentration of total protein in the blood was significantly higher in the 1×10^6 CFU/gm feed group ($P\leq0.05$), followed by the 1×10^8 CFU/gm feed group, compared to the other groups. Furthermore, the findings indicated that the concentration of albumin in the blood was significantly ($P\leq0.05$) higher in the 1×10^8 CFU/gm feed group, followed by the 1×10^4CFU /gm feed group, compared to the other groups. In addition, the results showed that the probiotic treatments caused a significant increase ($P\leq0.05$) in globulin concentrations in birds' serum in the fifth week, and similar parameters were obtained for the sixth week of their life. Table 5 illustrates that the high hormonal regulation of protein metabolism is probably the reason for the total protein rising in the treated groups, compared to the control group. Growth hormone leads to an increase in cellular protein synthesis, whereas glucocorticoids cause an increase in tissue protein breakdown, which indirectly influences protein metabolism (23). There is a clear increase in the body weights the treated groups, compared to the control group (Table 2), which might be due to the fact that the elevation of total protein in the blood indicates a higher rate of anabolism than catabolism in birds. The level of serum protein in the process of protein metabolism and degradation reflects the immune function *in vivo* and the state of protein metabolism. It was also found that the albumin and globulin in the total protein in the blood reflect the state of hepatic protein metabolism as a kind of nutritional response in birds (24). However, this study showed that probiotic-treated groups had a significantly higher level of the total serum protein, compared to the control group, which can improve whole body protein anabolism in birds. It is also important to mention that the probiotic *B. subtilis* BSW strain, analyzed in this study, holds a significant inhibitory impact on the aflatoxin B1 produced by *Aspergillus flavus*, reported by Al-Saad, Al-Badran (25). This toxin may cause a significant decrease in the growth rate and feed conversion ratio when present in the diet. It can also render birds highly susceptible to disease and mortality (26). Therefore, such features makes *B. subtilis* BSW a promising option to be used as a probiotic agent in addition to the advantages demonstrated throughout the present study.

5. Conclusion

It is concluded that the dietary supplementation of *B. subtilis* BSW in the diet of broiler chickens significantly improved their growth performance, compared to the control group. Instead, the treated groups exhibited a substantial increase in the total serum protein, serum albumin, and serum globulin, compared to the control group. These findings suggest that the *B. subtilis* BSW strain possesses probiotic properties, making it a suitable supplement for the poultry diet.

Authors' Contribution

Study concept and design: A. A. A. and B. A. A.
Acquisition of data: A. A. A.
Analysis and interpretation of data: W. A. A.
Drafting of the manuscript: M. Y. A.
Critical revision of the manuscript for important intellectual content: A. A. A., B. A. A. and W. A. A.
Statistical analysis: M. Y. A.
Administrative, technical, and material support: A. A. A., B. A. A. and W. A. A.

Ethics

All ethical standards related to bird care and husbandries were applied in this study and approved by the ethics committee of the University of Basrah, Basra, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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